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Micro Determination of Antibiotics Derivatives in Pharmaceutical Formulations

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Abstract:*The use of AHC reagent has not been reported for the estimation of the antibiotics. therefore in the present chapter, I have described a simple method for the determination of the following antibiotic drugs, such as Amoxicillin, Ampicillin, Cefixime, cloxacillin Sodium, Norfloxacin and Ornidazole in pure form and in their pharmaceutical preparations like Novamox-DT(Tab), Blumox-DT (Tab), Cincillin Introduction Rajeev Singh Baghel : 50 (Inj), roscillin (Inj), Cefexy (Tab) Hifen (tab), Klox (Inj), Neoclox (Tab), Uflox (Tab), Utibid (Tab), Giro (Tab) and Ornilox (Tab).*

Background*: The word antibiotic comes from the Greek antibiosis, which is made up from anti meaning "against" bios means "life" (i.e., against life) was introduced by the French bacteriologist. Vieillemin in 1877 when Louis Pasteur and Robert Koch observed that an airborne bacillus could inhibit the growth of Bacillus anthracis. These drugs were later renamed antibiotics by Selman Waksman, an American microbiologist in 1942.In 1939 Edwardchain and Heward Florey further studied penicillin and latter carried out trials of penicillin on humans (with what were deemed total bacterial infections) Fleming, florey and chain shared the Nobel Prize in 1945 for this work which shared in the era of antibiotics.*

Materials and Methods*: Aliquots containing 1-5mg of the sample were taken in 100ml stoppered conical flask followed by the addition of 5ml AHC (0.1M) reagent, prepared in 0.5N-HNO3. The reaction mixture was shaken well and allowed to react for required reaction time at room temperature (25-30ºC). The unconsumed Ce(IV) was titrated against 0.025M FAS solution using two drops of ferroin indicator (0.001M). A blank experiment was also performed under identical conditions using all the reagents except the sample. The amount of AHC consumed for the sample was calculated with the difference in the titre values of ferrous ammonium sulphate solution for blank and actual experiments.*

Results*: The use of AHC reagent has not been reported for the estimation of the antibiotics. therefore in the present chapter, I have described a simple method for the determination of following antibiotic drugs, such as Amoxicillin, Ampicillin, Cefixime, cloxacillin Sodium, Norfloxacin and Ornidazole in pure form and in their pharmaceutical preparations like Novamox-DT(Tab), Blumox-DT (Tab), Cincillin Introduction Rajeev Singh Baghel : 50 (Inj), roscillin (Inj), Cefexy (Tab) Hifen (tab), Klox (Inj), Neoclox (Tab), Uflox (Tab), Utibid (Tab), Giro (Tab) and Ornilox (Tab).*

Conclusion:*The use of AHC reagent has not been reported for the estimation of the antibiotics. therefore in the present chapter, I have described a simple method for the determination of following antibiotic drugs, such as Amoxicillin, Ampicillin, Cefixime, cloxacillin Sodium, Norfloxacin and Ornidazole in pure form and in their pharmaceutical preparations like Novamox-DT(Tab), Blumox-DT (Tab), Cincillin Introduction Rajeev Singh Baghel : 50 (Inj), roscillin (Inj), Cefexy (Tab) Hifen (tab), Klox (Inj), Neoclox (Tab), Uflox (Tab), Utibid (Tab), Giro (Tab) and Ornilox (Tab).*

Key Word*: Micro Determination, Antibiotic Drugs*

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I. Introduction

The word antibiotic comes from the Greek antibiosis, which is made up from anti meaning "against" bios means "life" (i.e., against life) was introduced by the French bacteriologist. Dieleman in 1877 when Louis Pasteur and Robert Koch observed that an airborne bacillus could inhibit the growth of Bacillus anthracis. These drugs were later renamed antibiotics by Selman Waksman, an American microbiologist in 1942.

In 1939 Edwardchain and Heard Florey further studied penicillin and latter carried out trials of penicillin on humans (with what were deemed total bacterial infections) Fleming, Florey and chain shared the Nobel Prize in 1945 for this work which shared in the era of antibiotics.

The first antibiotic was discovered by Sir Alexander Fleming in 1928. The development of antibiotics is probably the largest advance in medicine in 20th century and has saved millions of live worldwide from infections such as tuberculosis. The first antibiotic was penicillin. Such penicillin related antibiotics as ampicillin, amoxicillin, and benzylpencillin are widely used today to treat a variety of infections. When

antibiotics were isolated in the mid-twentieth century, they were widely hailed as "wonder drugs" and indeed, formerly life-threatening infections could now be easily cured within a few days. Antibiotic drugs are used in the treatment of an infections caused by bacteria 1-2 They target only bacteria i.e.; they do not attack organisms such as fungi or viruses. Antibiotics work best when the amount of medicine is body is kept at a constant level. Antibiotic drugs will not work for colds, flu, or other viral infections. Due to their great medicinal importance, a wide variety of analytical methods have been reported for determination of antibiotics, a wide variety fo analytical methods have been reported for determination of antibiotics in pure form and in their Pharmaceutical and in biological fluids. These methods include spectrophotometry atomic absorption spectroscopy Fluorimetry[7-11] capillary chromatography[12-13] Potentiometric[14-15] and polarographic 16-18 methods.

II. Material and Methods

Chemically Amoxicillin in (2S, 5R, 6R) - 6 - {[(2R)-2amino-2-(4-hydoxyphenyl)acety]-amino}-3, 3-dimethyl 1-7- oxo-4 thia-1-azabicyclo [3.2.0]heptanes-2 carboxylic acid. Structurally it is represented as: Molecular formula: C16H19N3O5S Molecular weight: 387.4 Melting point: 1940C It is off white crystalline powder, freely soluble in sulphuric acid and partially soluble in cold and hot water.

Amoxicillin is an antibiotic of the penicillin group of drugs. It is used to treat infections due to organism that are susceptible to the effects of Amoxicillin. Common infections that amoxicillin is used for include infections of the middle ear, tonsils, larynx (laryngitis). bronchi (bronchitis)] lungs (pneumonia), urinary tract and skin. It is also used to treat gonorrhea.

Aliquots containing 1-5mg of the sample were taken in 100ml stoppered conical flask followed by the addition of 5ml AHC (0.1M) reagent, prepared in $0.5NHNO_3$. The reaction mixture was shaken well and allowed to react for required reaction time at room temperature (25-30ºC). The unconsumed Ce(IV) was titrated against 0.025M FAS solution using two drops of ferroin indicator (0.001M). A blank experiment was also performed under identical conditions using all the reagents except the sample. The amount of AHC consumed for the sample was calculated with the difference in the titre values of ferrous ammonium sulphate solution for blank and actual experiments. the recovery of the sample was calculated with theamount of AHC consumed for the sample.For every sample percentage error, coefficient of variation and standard deviation were calculated.

III. Resultand Discussion

For testing the quantitative validity of reaction, Amoxicillin was taken as test sample. Different amount of sample (1-5mg) were allowed to react with varying concentrations of Ammonium Hexanitratocerate (IV) at room temperature (25-30ºC) for different intervals of time. The unconsumed AHC was back titrated perimetrically. A blank experiment was also run under identical conditions using all the reagents except the sample. the difference in the titre values of ferrous ammonium sulphate consumed for blank and actual experiments was used to calculate the amount of the sample present in a particular experiment. the stoichiometry of the reaction was established for each sample and a possible course of reaction was also suggested. On the basis of the reaction conditions developed for amoxicillin the determination of other compounds in the pure form and in their pharmaceutical preparations were done.

In order to develop suitable reaction condition for the determination of above antibiotics with AHC reagent, the effect of different variables such as reaction time, reagent concentration, acidic medium and reaction temperature were studied and calculated.

Effect of reaction time:

Keeping the amount of Amoxicillin, the concentration of AHC reagent as constant, the reaction time was varied form 1-25 minutes. Aliquots containing 5mg of Amoxicillin were taken in 100ml stoppered conical flask and 5ml of 0.1M AHC reagent prepared and 10ml of 4Msulphuric acid was added to it. Now

the reaction mixture was shaken well and allowed to react at room temperature for 1-25 minutes. After the reaction was over, the unconsumed AHC was determined by back titrating the reaction mixture against standardized ferrous ammonium sulphate (0.025M) solution using ferroin as indicator. The percentage recovery of the sample does not change after certain reaction time. Therefore further estimation was done on the same reaction time (Table-3). Similar experiments were performed with other samples as well. It was observed that all other antibiotics require the same reaction time (15 minutes) to complete the reaction except cloxacillin sodium and Norfloxacin which requires only 10 minutes.

Effect of concentration of sulphuric acid:

Keeping the reaction time, amount of Amoxicillin and concentration of AHC (0.1M) constant, the concentration of sulphuric acid was varied from (1-7M) and the results were noted in (Table-9). Results show that the best recovery of the samples was obtained at 4M concentration of sulphuric acid. To ascertain the exact amount of 4Msulphuric acid needed for the reaction, some variations in the valume were done (Table-10), accurate results

were obtained at 10Ml of the acid. similar results were obtained in case of other antibiotics. Thus, for completing the reaction and getting accurate results 10ml sulphuric acid was recommended for the experiment.

Effect of concentration of AHC:
Keeping the reaction time, amount of Amoxicillin and concentration of sulphuric acid as constant, the effect of varying concentration of AHC was studied. 5mg of the sample was allowed to react with 5ml of varying concentration (0.01-0.1M) of AHC. The unconsumed AHC was back titrated with FAS (0.025M) solution using ferroin indicator and the recovery of the sample was calculated. It was found that the best results wereobtained at 0.1M concentration of AHC. the concentration of reagent less than 0.1M gives higher percentage of error and low recovery (table-11). The reason for this is due to incomplete reaction of the reagent with the sample. The higher concentration than 0.1M gives no significant advantage in percentage recovery. therefore, the higher concentration of the reagent was avoided. Variation in the volume of 0.1M AHC was also studied. It was observed that 5ml of 0.1M AHC gives accurate result. Thus for completing the reaction, getting accurate results and also avoiding the wastage of the reagent, 5ml of 0.1M AHC was recommended for the experiment. In the similar way the studies of different variables were also done with Amoxicillin.

Effect of temperature:
Keeping all other conditions constant, the reaction temperature was varied from 50C onwards and the recovery of Amoxicillin was calculated (Table-13). It was observed that the reaction was completed within 15 minutes at room temperature (25-300C). The heating of the reaction mixture directly in flame, hot plate or boiling water bath gives inaccurate results. It may be due to decomposition of reagent at high temperature. Although the reaction is completed at room temperature, but the experiment were also carried out at lower temperature up to 50C. In this case also a decrease in recovery of the sample was noted. It shows that the reaction is slow at lower temperature. Thus for the estimation of Amoxicillin a reaction is slow at the lower temperature. Thus for the estimation of Amoxicillin a reaction temperature of 25-300C was maintained. Such experiments were carried out with all other samples and the recovery was noted. It was observed that the reaction recommended temperature was suitable for all other antibiotics e.g. Ampicillin, Cefixime, Cloxacillin Sodium, Norfloxacin and Ornidazole.

Stoichiometry of the reaction:
Aliquots containing 5mg of the sample was taken in a 100ml stoppered conical flask and 5mL of 0.1m AHC reagent, prepared in 0.5N nitric acid was added. The reaction mixture was shaken thoroughly and allowed to react for required reaction time at room temperature (25-30ºC) and then added 10Ml of 4M H2SO4solution. the unconsumed Ce(IV) was titrated against with 0.025M FAS solution using two drops of ferroin indicator (0.001M). A blank experiment was also run under identical conditions using all the reagents except the sample. Number of moles of the AHC consumed for Amoxicillin was calculated with the difference in the titre values of FAS solution consumed for blank and actual experiments (table-1). Similar experiments were performed for Ampicillin, Cefixime, Cloxacillin sodium, Norfloxacin and Ornidazole. It was observed that the molar ratio of above drugs with AHC reagent was 1:6, 1:4, 1:4, 1:6 and 1:4 respectively.

It has already been mentioned, the stoichiometric ratio between AHC reagent and antibiotics such as Amoxicillin (1:6), ampicillin (1:6), Cefixime (1:4), cloxacillin sodium (1:4) Norfloxacin (1:6) and Ornidazole (1:4) in pure form and in their pharmaceutical preparations. this ratio remains constant even under varying reaction conditions. It has been observed in the experiments on variation of reaction time a particular reaction time is needed for completion of the reaction and for concordant and accurate results. The reaction time varies from one compound to another. At the reaction time lesser than the described (table-3), inaccurate results are obtained because of incomplete reaction. an increase in the prescribed reaction time does not give any change in percentage recovery of the sample.

The use of sulfuric acid as a proper reaction medium has also been studied. H_2SO_4 gives quantitative and stoichiometric results with Amoxicillin and Ampicillin. the same results were obtained in the case of other samples. Reaction was also carried out in the absence of H_2SO_4. In this case, it was found that the reaction is slow and the percentage of error is very high. So, it was observed that a proper reaction medium is necessary for the accurate results. After variation in the concentration and volume of sulfuric acid, it was observed that the use of 10mL sulfuric acidwas necessary for suitable reaction medium.

AHC is the main active agent, which reacts with antibiotic drugs. As indicated, the 5mL of 0.1M AHC was sufficient for all the samples for accurate results. reaction was also carried out at lower and higher (0.01-0.1M) concentration at variable volume of AHC. in this case, it was observed that the concentration and volume than the prescribed under reaction conditions gives low recovery because of insufficient reagents. Higher concentration and volume do not give any improvement over the results. Therefore, prescribed concentration and volume of the AHC reagent was used. The effect of temperature has also been studied. It is observed that

the results improve with increase in reaction temperature. the best recovery was obtained at room temperature (25-30ºC). An increase in the reaction temperature above gives inaccurate results (table-13). At a lower temperature (20-25ºC), it is decreased that the reaction is very slow and needs more reaction time. It gives higher percentage of error.

The stoichiometry of the reaction for amoxicillin was established. Aliquots containing 5mg of the sample was taken in a 100ml stoppered conical flask and 5mL AHC reagent, prepared in 0.5N nitric acid was added. The reaction mixture was shaken thoroughly and allowed to react for required reaction time at room temperature (25-30ºC) and then added 10mL of $4MH_2SO_4$solution. the unconsumed Ce(IV) was titrated against with 0.025M FAS solution using two drops of ferroin indicator (0.001M). A blank experiment was also run under identical conditions using all the reagents except the sample. Number of moles of the AHC consumed for Amoxicillin was calculated with the difference in the titre values of FAS solution consumed for blank and actual experiments. Similar experiments were performed for Ampicillin, Cefixime, Cloxacillin sodium, Norfloxacin and Ornidazole. It was observed that the molar ratio of above drugs with AHC reagent was 1:6, 1:4, 1:4, 1:6 and 1:4 respectively.

IV. Conclusion

The development of antibiotics is probably the largest advance in medicine in 20th century and has saved millions of live worldwide from infections such as tuberculosis.On the basis of oxidation pattern of these compounds and literature available a possible course of reaction for Amoxicillin, Ampicillin, cloxacillin sodium, cefixime and Ornidazole with AHC reagent may be suggested.

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